

WHAT IS CLAIMED IS:

1        1.        A method for making an infectious adenovirus which comprises contacting a cell with or  
2        introducing into a cell:

3        (a)        a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
4        of intermolecular recombination, are insufficient to encode an infectious, replicable  
5        or packageable adenovirus; and

6        (b)        a second nucleic acid sequence encoding adenovirus sequences which, in the absence  
7        of adenoviral replication factors provided in trans or intermolecular recombination  
8        with said first nucleic acid sequence, are insufficient to encode an infectious,  
9        replicable or packageable adenovirus;

10       provided that said first and said second nucleic acid sequences each comprise a head-to-head  
11       ITR junction and sufficient overlapping adenoviral nucleic acid sequences such that  
12       homologous recombination may occur between said first and said second nucleic acid  
13       sequences; whereby said first and said second nucleic acids recombine to form said  
14       infectious adenovirus.

1       2.        The method according to claim 1 wherein said first nucleic acid sequence is a plasmid  
2        containing a circularized adenovirus DNA molecule encoding adenovirus sequences which,  
3        in the absence of intermolecular recombination, are insufficient to encode an infectious,  
4        replicable or packageable adenovirus.

1 3. The method according to claim 2 wherein said plasmid includes a bacterial origin of DNA  
2 replication, an antibiotic resistance gene for selection in bacteria, a deletion or modification  
3 in E1 that renders the adenoviral sequences insufficient to form infectious virus, and  
4 combinations thereof.

1 4. The method according to claim 2 wherein said adenovirus DNA has a deletion of an  
2 adenoviral packaging signal.

1 5. The method according to claim 4 wherein said adenovirus DNA comprises (i) a deletion of  
2 or (ii) a modification in, of an adenoviral gene selected from the group consisting of  
3 adenoviral E1 sequences extending beyond said packaging signal, adenoviral fibre gene  
4 sequences, adenoviral E3 gene sequences, adenoviral E4 gene sequences, and combinations  
5 thereof.

1 6. The method according to claim 1 wherein said second nucleic acid sequence is a plasmid  
2 comprising:

- 3 (i) said head-to-head ITR junction, and a packaging signal contained within the leftmost  
4 approximately 350 nt of the adenovirus genome; and,  
5 (ii) a polycloning site or a foreign DNA or an expression cassette.

1        7.        The method according to claim 6 wherein said second nucleic acid sequence is selected from  
2                the group consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117,  
3                pDC118, and identifiable combinations thereof which, as optionally needed, undergo  
4                additional modification to provide a head-to-head ITR junction.

1        8.        A recombinant adenovirus vector system comprising:

2                (a)        a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
3                of intermolecular recombination, are insufficient to encode an infectious, replicable  
4                or packageable adenovirus, said first nucleic acid sequence comprising a head-to-  
5                head ITR junction and sufficient overlapping adenoviral nucleic acid sequences such  
6                that homologous recombination with sequences in a second nucleic acid sequence  
7                may occur;

8                (b)        the second nucleic acid sequence, encoding adenovirus sequences which, in the  
9                absence of adenoviral replication factors provided in trans or intermolecular  
10               recombination with said first nucleic acid sequence, are insufficient to encode an  
11               infectious, replicable or packageable adenovirus, said second nucleic acid sequence  
12               comprising a head-to-head ITR junction and sufficient adenoviral sequences to  
13               permit homologous recombination with said first nucleic acid sequence;

14               whereby said first and said second nucleic acids homologously recombine to form said  
15               infectious adenovirus.

1 9. The recombinant adenovirus vector system of claim 8 wherein said second nucleic acid  
2 sequence is a plasmid selected from the group consisting of pDC111, pDC112, pDC113,  
3 pDC114, pDC115, pDC116, pDC117, pDC118, and identifiable combinations thereof which,  
4 as optionally needed, undergo additional modification to provide a head-to-head ITR  
5 junction.

1 10. The recombinant adenovirus vector system of claim 8 wherein said cell further expresses  
2 adenoviral E1.

1 11. The recombinant adenovirus vector system of claim 8 wherein said first plasmid and said  
2 second plasmid are cotransfected into said cell to produce an infectious virus vector  
3 comprising a left end, a polycloning site, foreign DNA, or an expression cassette derived  
4 from said second plasmid, joined to the remaining portion of the viral DNA derived from  
5 said first plasmid.

1 12. The recombinant adenovirus vector system of claim 8 wherein second nucleic acid sequence  
2 is a plasmid selected from the group consisting of pDC111, pDC112, pDC113, pDC114,  
3 pDC115, pDC116, pDC117, pDC118, and identifiable combinations thereof which, as  
4 optionally needed, undergo additional modification to provide a head-to-head ITR junction.

1 13. A kit for construction of recombinant adenovirus vectors comprising:

- 2 (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
3 of intermolecular recombination, are insufficient to encode an infectious, replicable  
4 or packageable adenovirus, said first nucleic acid sequence comprising a head-to-tail  
5 ITR junction and sufficient adenoviral sequences to permit homologous  
6 recombination with similar sequences in a second nucleic acid sequence;
- 7 (b) the second nucleic acid sequence encoding adenovirus sequences which, in the  
8 absence of adenoviral replication factors provided in trans or intermolecular  
9 recombination with said first nucleic acid sequence, are insufficient to encode an  
10 infectious, replicable or packageable adenovirus, said second nucleic acid sequence  
11 comprising a head-to-head ITR junction and sufficient adenoviral sequences to  
12 permit homologous recombination with similar sequences in said first nucleic acid;  
13 and
- 14 (c) a cell wherein, when said component (a) and said component (b) are cotransfected  
15 and recombined through homologous recombination, an infectious recombinant  
16 adenovirus vector is produced.

1 14. The kit according to claim 13 wherein said component (b) is selected from the group  
2 consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, pDC118,  
3 and identifiable combinations thereof which, as optionally needed, undergo additional  
4 modification to provide a head-to-head ITR junction.

1 15. The kit according to claim 13 wherein said cell of (c) is a 293 cell.

- 1 16. The recombinant adenovirus vector system according to claim 8 wherein said first nucleic  
2 acid sequence comprises a deletion in the adenoviral fibre gene.
- 1 17. The recombinant adenovirus vector system according to claim 8 wherein an adenoviral gene  
2 mutation is rescued into said adenoviral vector recombinant.
- 1 18. The recombinant adenovirus vector system according to claim 17 wherein said adenoviral  
2 gene mutation rescued into said adenoviral vector recombinant is a mutation in the  
3 adenoviral fibre gene, the adenoviral E4 gene, the adenoviral E3 gene, or combinations  
4 thereof.
- 1 19. A cell into which has been introduced a first vector selected from the group consisting of  
2 pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, pDC118, and identifiable  
3 combinations thereof which, as optionally needed, undergo additional modification to  
4 provide a head-to-head ITR junction.
- 1 20. A method of vaccinating or administering gene therapy to a recipient in need of such  
2 treatment which comprises administering to said recipient an effective amount of an  
3 adenovirus produced by homologous recombination of:  
4 (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
5 of intermolecular recombination, are insufficient to encode an infectious, replicable

6 or packageable adenovirus, said first nucleic acid sequence comprising a head-to-tail  
7 ITR junction and sufficient adenoviral sequences to permit homologous  
8 recombination with similar sequences in a second nucleic acid sequence;  
9 (b) the second nucleic acid sequence encoding adenovirus sequences which, in the  
10 absence of adenoviral replication factors provided in trans or intermolecular  
11 recombination with said first nucleic acid sequence, are insufficient to encode an  
12 infectious, replicable or packageable adenovirus, said second nucleic acid sequence  
13 comprising a head-to-head ITR junction and sufficient adenoviral sequences to  
14 permit homologous recombination with similar sequences in said first nucleic acids;  
15 wherein said first and said second nucleic acid sequences, in combination and following said  
16 homologous recombination, result in production of an infectious adenovirus for use in said  
17 method of vaccinating or administering gene therapy to a recipient in need thereof.

1 21. The method according to claim 20 wherein said second nucleic acid is selected from the  
2 group consisting of pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117,  
3 pDC118, and identifiable combinations thereof, which, as optionally needed, undergo  
4 additional modification to provide a head-to-head ITR junction.

1 22. An improved adenovirus vector system comprising two plasmids, each with sufficient similar  
2 adenoviral sequences to permit homologous recombination with one another, and neither of  
3 which alone comprises sufficient adenoviral sequences to produce infectious adenovirus  
4 when introduced into a cell but which, when both plasmids are introduced into a cell,

5 recombine to form an infectious recombinant adenovirus, the improvement comprising  
6 inclusion of a head-to-head ITR junction in both of the plasmids.

1 23. A two-plasmid system for making an infectious adenoviral vector wherein each plasmid  
2 alone comprises insufficient adenoviral sequences to encode an infectious adenoviral vector  
3 wherein, upon homologous recombination, an infectious adenoviral vector is produced,  
4 provided that each plasmid of said two-plasmid system comprises (a) a head-to-head ITR  
5 junction, and (b) sufficient similar adenoviral sequences to permit said homologous  
6 recombination with one another.